Charge-coupled imagers for time-resolved macromolecular crystallography

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There exists considerable promise for the use of charge-coupled device (CCD) imagers in the fast recording of parts of macromolecular crystal Laue diffraction patterns. As part of this development CCD tests have been made with direct detection of Laue patterns from a small molecule test crystal and a protein crystal. Merging *R* factors (on intensity), for strong reflections, of 3% have been obtained. A time-slicing scheme for a CCD camera is discussed based on the stacking of slices held in storage in the CCD in the submillisecond time resolution range.

I. INTRODUCTION

Time-resolved macromolecular crystallography has been made possible with the advent of synchrotron x radiation (SR). The Laue method is preferred because a stationary crystal sample is used and exposure times are very short. At Daresbury the use of a focused white beam on Station 9.5 of the wiggler line has resulted in millisecond and submillisecond exposure times for a protein crystal for photographic film and image plates, respectively.[1]

A time-resolved experiment[2] involves the key stages of reaction initiation, reaction monitoring, and full data set acquisition.[3] Reaction monitoring can be either via spectrophotometry on the crystal, if it contains a suitable chromophore, and/or real-time monitoring of the Laue diffraction pattern itself. As a diagnostic, only a small part of the Laue pattern is required to assess when reaction intermediates build up in the crystal. Currently available CCDs are well suited to the monitoring of a part of a Laue pattern. Indeed since 50 Laue spots or so only occupy about 10 mm^2 in a protein crystal Laue pattern, under typical conditions, then a CCD of size 17×26 mm^2 can actually accommodate a stack of time slices by masking off a large area of the CCD from the diffraction pattern.

This paper documents our progress with the design and implementation of a CCD system for reaction monitoring in a crystal in real time via part of the available Laue diffraction pattern. The use of a CCD with detection directly via the silicon allows a fine point spread factor to be realized and avoids long decay time processes associated with phosphor coupled systems. However, in order to allow for a variety of time-resolved experiments, slow and quick, and to facilitate comparisons between direct and phosphor coupled data collection strategies we have an indirect detection head available. Figure 1(a) shows an overall layout of the experimental configuration.

II. THEORY

Laue diffraction patterns are well characterized now. A large fraction of the population of Laue spots are single rather than multiple reflection spots.[4] Hence, the positioning of a CCD detector to pick up part of a Laue pattern is guaranteed a high score of single component spots. The angular distribution of Laue spots and their associated wavelengths has been dealt with in a recent paper;[5] there is a strong correlation of wavelength with angle so that the positioning of the CCD with respect to the direct beam can be selected to optimize the wavelength for which the measured spots yield their strongest signal.

III. EXPERIMENT

Basic tests of a CCD have been made, with the direct detection mode, using a device from EEV with a deep depletion region (30 μm), overall size 8×6 mm^2, pixel size 22.5-μm square. An earlier paper[6] reported a measurement of the DQE at 17.5 keV (Mo $K\alpha$) of 5.6% for this device. We now report here several further measurements on it. Reproducibility tests were made on strong Laue reflections, measured repeatedly from a stable, organic, small molecule crystal.[7] Over a period of exposure time of several seconds, ~16 measurements yielded a merging *R* factor, an intensity of 3%. In another experiment on a protein crystal, carbonic anhydrase II,[8] a sequence of exposures

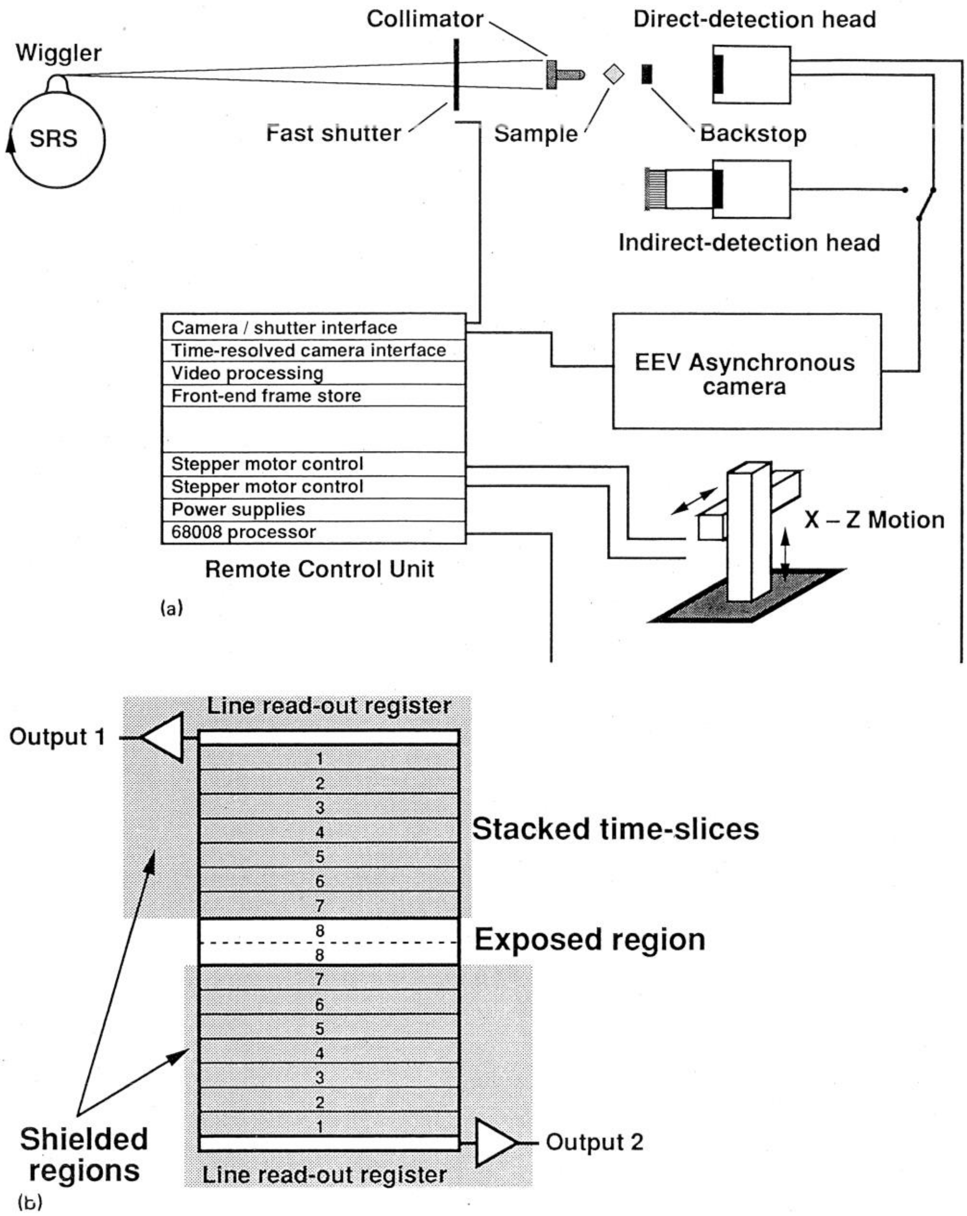

FIG. 1. (a) Schematic of experimental arrangement. (b) Outline mode of operation.

was taken. The crystal withstood 1.92 s of irradiation in the unfocused white beam from the Daresbury wiggler. During this time three high-quality images were obtained of the Laue pattern (i.e., each of 0.64-s duration) before radiation damage to the sample was significant.

IV. FUTURE

The time-slicing camera will use an EEV CCD 05-20 Series device, with high resistivity substrate, with twin high-speed readout (up to 15-MHz pixel clock) amplifiers. *A d* convertors, with digital video processing, will feed directly to the image store and processor. Precision movable shielding will permit either full-frame, frame-transfer (at conventional TV rates—for on the fly viewing), or time-slicing modes of operation. In the time-slicing mode, a number of stacked slices are to be held in split analog storage in the CCD [Fig. 1(b)]. The minimum transfer time of each slice into this storage will be of the order of 220–860 μs. This provides a good match to the expected exposure times, with the device, on the focused wiggler beam. Hence, millisecond time resolution will be possible for reaction monitoring of events in a crystal.

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